

## FastStart Technology for Hot-Start PCR Is Now Available for More Real-Time PCR Instruments

### FastStart TaqMan<sup>®</sup> Probe Master

The FastStart TaqMan<sup>®</sup> Probe Master is a ready-to-use reagent mix that simplifies the preparation of samples for quantitative PCR (qPCR) and two-step quantitative reverse transcription PCR (qRT-PCR). In combination with a real-time PCR instrument, suitable PCR primers and Hydrolysis Probe (e.g., from the Universal ProbeLibrary), the FastStart TaqMan<sup>®</sup> Probe Master allows very sensitive detection and quantification of defined DNA sequences. This master mix is optimized for use with most real-time PCR instruments other than the LightCycler<sup>®</sup> 2.0 System Instruments (Table 1).

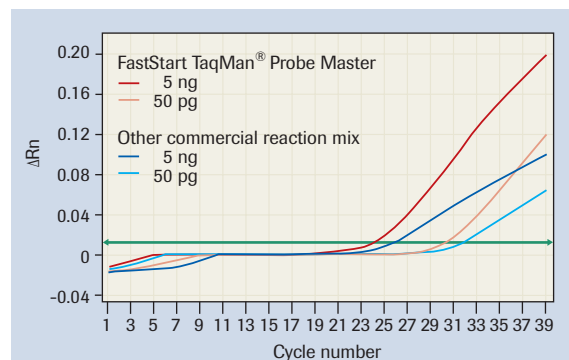
The double-concentrated master mix contains all reagents (except primers, probe, and template) needed for running quantitative, real-time PCR assays in the Hydrolysis Probe detection format. It is available in two formulations: one with and one without the Rox reference dye.

#### Advantages and Applications

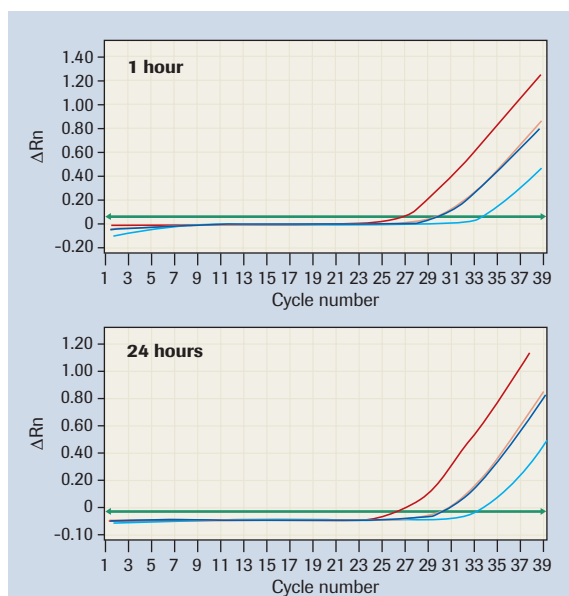
- ➔ Use the master mixes for the amplification and detection of any DNA or cDNA target.
- ➔ Amplify targets up to 500 bp.
- ➔ Benefit from convenience and ease-of-use: The addition of MgCl<sub>2</sub> to the reaction mixture is not required, thereby avoiding time-consuming optimization steps.
- ➔ Visualize amplification products on agarose gels.
- ➔ Avoid false-positive signals from carry-over contamination. The mix contains dUTP and may be used with uracil-DNA glycosylase.

The FastStart TaqMan<sup>®</sup> Probe Master outperforms master mixes from other suppliers especially when used in combination with the Universal ProbeLibrary (Figure 1). The

mix – supplemented with primers, probes, and template – is stable for at least 24 hours at room temperature (Figure 2). This is especially important when robotics are used to pipette qPCRs, as the automation requires a stable reaction mix over a specific period of time.



**Figure 1: Two-step real-time RT-PCR of the apolipoprotein-E gene using probe no. 72 from the Universal ProbeLibrary.**



**Figure 2: Stability.** The stability of a complete reaction mix was assessed at room temperature using the FastStart TaqMan<sup>®</sup> Probe Master after 1 hour and 24 hours on an Applied Biosystems 7500 Real-Time PCR System.

**Table 1: FastStart master mixes are optimized for most real-time PCR instruments**

Assay	Instrument	
	LightCycler <sup>®</sup> 2.0 System Instruments	Other Instrument (e.g., ABI 7500 Real-Time PCR System)
Binding Dye-based	LightCycler <sup>®</sup> FastStart DNA Master <sup>PLUS</sup> SYBR Green I	FastStart SYBR Green Master
Probe-based	LightCycler <sup>®</sup> TaqMan <sup>®</sup> Master	FastStart TaqMan <sup>®</sup> Probe Master

## FastStart SYBR Green Master

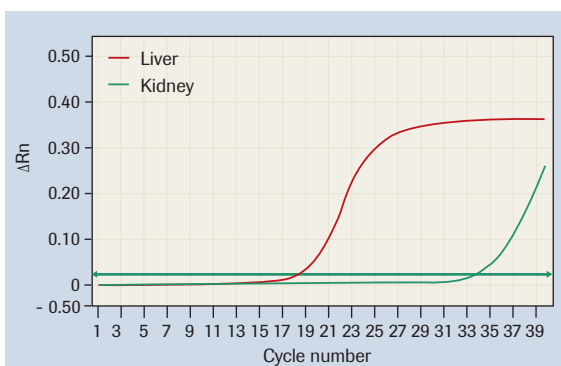
If – after considering the value of the Universal ProbeLibrary/FastStart TaqMan® Probe Master combination – you still want to perform a SYBR Green I-based qPCR assay, choose the new FastStart SYBR Green Master. This ready-to-use, double-concentrated master mix contains all the necessary reagents (except primers and template) and is suitable for quantitative, real-time DNA detection assays whenever you perform qPCR and two-step qRT-PCR assays in the SYBR Green I detection format. It is designed for universal use: consequently, GC-rich and GC-poor templates can be utilized equally well. This master mix is optimized for use with most real-time PCR instruments other than the LightCycler® 2.0 System Instruments.

### Advantages and Applications

- ➔ Improve PCR sensitivity and specificity. Rely on this mix's FastStart Taq DNA Polymerase to minimize the formation of nonspecific amplification products through hot-start PCR. Simply heat activate this chemically modified enzyme, which is completely inactive at the lower temperatures at which primers often bind nonspecifically (prior to the first cycle).
- ➔ Avoid over-estimation of qPCR results. Eliminate nonspecific amplification products and primer-dimers that would increase the amount of quantified SYBR Green I.
- ➔ Use any block-based real-time PCR instrument. Choose from two formulations, one that contains the Rox reference dye and one without Rox. Perform qPCR on any platform other than the LightCycler® 2.0 System Instruments.
- ➔ Save time and effort in qPCR preparation. Rely on this easy-to-use 2x master mix to eliminate the need to mix components, titrate MgCl<sub>2</sub>, or perform other time-consuming optimization steps.
- ➔ Prevent false positives from carry-over contamination. Add Uracil-DNA Glycosylase to degrade amplification products from previous PCRs with this mix, which contains dUTP.

### Combination with the Transcriptor First Strand cDNA Synthesis Kit

The combination of these master mixes with the Transcriptor First Strand cDNA Synthesis Kit allows for superior results in two-step qRT-PCR. The Transcriptor First Strand cDNA Synthesis Kit is specifically designed



**Figure 3: Quantification of the tubulin gene in different rat tissues using FastStart SYBR Green Master on an Applied Biosystems 7500 Real-Time PCR System.**

Reverse Transcription of mRNA was performed using Transcriptor First Strand cDNA Synthesis System.

and function tested for qPCR, and is efficient with all real-time PCR instruments:

- ➔ Achieve accurate linear quantification over at least a 10<sup>8</sup>-fold range of input RNA (*in vitro* transcripts).
- ➔ Analyze genes with very low or extremely high expression levels.
- ➔ Use the kit's unique anchored-oligo(dT)<sub>18</sub> primer, which binds at the beginning of the poly(A) tail rather than mispriming at an internal site within the tail.

Figure 3 shows the results of quantification of the tubulin gene in different mouse tissues using the FastStart SYBR Green Master in combination with the Transcriptor First Strand cDNA Synthesis System. ■

Product	Pack Size	Cat. No.
<b>FastStart TaqMan® Probe Master (Rox)</b>	2 x 1.25 ml, 100 reactions (50 µl)	04 673 450 001
	10 x 1.25 ml, 500 reactions (50 µl)	04 673 468 001
	10 x 5 ml, 2000 reactions (50 µl)	04 673 476 001
<b>FastStart TaqMan® Probe Master</b>	2 x 1.25 ml, 100 reactions (50 µl)	04 673 409 001
	10 x 1.25 ml, 500 reactions (50 µl)	04 673 417 001
	10 x 5 ml, 2000 reactions (50 µl)	04 673 433 001
<b>FastStart SYBR Green Master (Rox)</b>	4 x 1.25 ml, 200 reactions (50 µl)	04 673 514 001
	10 x 5 ml, 2000 reactions (50 µl)	04 673 522 001
<b>FastStart SYBR Green Master</b>	4 x 1.25 ml, 200 reactions (50 µl)	04 673 484 001
	10 x 5 ml, 2000 reactions (50 µl)	04 673 492 001
<b>Transcriptor First Strand cDNA Synthesis Kit</b>	1 kit (50 reactions)	04 379 012 001

